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Low genetic diversity in a widespread whistling alien: A comparison of *Eleutherodactylus johnstonei* Barbour, 1914 (Eleutherodactylidae) and congeners in native and introduced ranges

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Abstract

There is no clear empirical evidence to support the general assumption that genetic diversity favours successful invasions. Many invading species disperse and establish successfully despite low genetic diversity, a phenomenon known as the genetic paradox of biological invasion. Model systems that allow comparison of genetic patterns between exotic and native source populations are still scarce. This is particularly true for amphibians. Here we compare genetic patterns of the widely introduced Johnstone's Whistling Frog, *Eleutherodactylus johnstonei*, with its successful alien congener *E. antillensis* and the single island endemic *E. portoricensis*. Genetic diversity and population differentiation in native and introduced populations of the three taxa were inferred from mitochondrial D-loop sequences (235 bp). Our results reveal that exotic populations of the two alien taxa, *E. johnstonei* and *E. antillensis*, are not only genetically impoverished due to founder effects, but that, moreover, their native range source-populations exhibit low genetic diversity and inter-population differentiation in the first place. Populations of the endemic *E. portoricensis*, on the other hand, are genetically more diverse and show marked inter-population differentiation. These observed

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genetic patterns are consistent with geological processes and invasion histories. We argue that the establishment success of the alien taxa in our model system is better explained by ecological factors and anthropogenic drivers than by genetic diversity. As these factors provide more parsimonious explanations, they should be given priority in management decisions. However, molecular studies with higher resolution are needed to fully test possible genetic and epigenetic components that could promote the invasion process.

Keywords
Alien amphibians, Anura, D-loop, genetic paradox, Lesser Antilles, population genetics

Introduction

Understanding the mechanisms of successful invasions is at the heart of invasion biology. More recently, the field has turned to molecular approaches that address their genetic basis (Bock et al. 2015). Introduced populations are often genetically impoverished as a result of strong founder effects that drive populations through genetic bottlenecks (Nei et al. 1975; Dlugosch and Parker 2008). Although intra-population genetic diversity is thought to be essential for successful invasion and establishment (Booy et al. 2000), several taxa with low genetic diversity have successfully established themselves in non-native areas (Allendorf and Lundquist 2003). This phenomenon is known as the genetic paradox of biological invasions (Allendorf and Lundquist 2003; Pérez et al. 2006). Yet, a genetic paradox is often simply assumed (Estoup et al. 2016) and differences in the genetic makeup between native and introduced populations are rarely tested systematically (e.g. Bradshaw et al. 2007; Stipoljev et al. 2021). However, this information is a prerequisite for tackling more complex questions with respect to the assumed correlation between genetic diversity, adaptive potential and invasion success.

Evidence from studies that compared genetic diversity of invasive taxa in their native and introduced ranges is ambiguous (Dlugosch and Parker 2008). Many successful invaders show few signs of genetic impoverishment in introduced populations (Tonione et al. 2011; Wellband et al. 2017, 2018; Negri et al. 2018), while others show very low genetic diversity across a wide exotic range (Harrison and Mondor 2011; Edelaar et al. 2015; Castillo et al. 2018). However, to investigate the true interaction between genetic diversity and successful invasions, comparisons are needed not only between exotic and native populations, but also between invasive and non-invasive congeners. Studies following this framework allow us to address the importance of the original genetic makeup of the source population in determining invasion success (Rollins et al. 2013; Romiguier et al. 2014; Trucchi et al. 2016; Baltazar-Soares et al. 2017). Here, we introduce a novel amphibian model system to test correlative patterns of genetic diversity and invasion success.

Robber Frogs of the genus *Eleutherodactylus* Duméril & Bibron, 1841 are a very diverse and species rich (206 recognised species) group of small to medium-sized direct developing frogs that have their distribution centre in the Antilles (Dugo-Cota et al. 2019; Frost 2021). Most species have very restricted ranges and can be considered single-

island or even micro-endemics restricted to small habitat patches on particular islands. However, a few species have succeeded in establishing themselves outside their native range (e.g. *E. antillensis*, *E. coqui*, *E. planirostris* and *E. martinicensis*). The most widely and successfully expanding species in the genus, and one of the most successful alien amphibians, is Johnstone's Whistling frog, *Eleutherodactylus johnstonei* Barbour, 1914. Today, it occurs on the majority of Caribbean islands and in many countries on the South American mainland (Kaiser et al. 2002; Ernst et al. 2011) as well as in Europe, where it is restricted to confined populations in greenhouses (Leonhardt et al. 2019; Moravec et al. 2020). Due to a lack of historic distribution data, it is difficult to unambiguously trace back the geographic origin of the species. Based on the cumulative historical and molecular evidence (Kaiser 1997; Censky and Kaiser 1999; Yuan et al. 2022), we here assume St. Lucia to be the most likely origin of exotic populations outside the Lesser Antilles.

In the present study we investigate the genetic diversity and haplotype distribution of *Eleutherodactylus johnstonei* across its assumed native range and in selected exotic populations. We compare these data with two congeneric species, *E. antillensis* (successful alien, native to Puerto Rico) and *E. portoricensis* (Puerto Rican endemic). We integrate extensive field and laboratory data sets for our focus taxon *E. johnstonei* with previously published data for *E. antillensis* and *E. portoricensis* to test the following assumptions. (1) *E. johnstonei* goes through genetic bottlenecks resulting in reduced genetic diversity in introduced populations compared to native populations. (2) Successful alien species in our model system (*E. johnstonei* and *E. antillensis*) are *a priori* genetically more diverse with respect to their non-expanding congener (*E. portoricensis*). We discuss the results in the light of the genetic paradox of biological invasions and with respect to the invasion history and ecology of the species considering previously proposed expansion scenarios.

Methods

Within our analytical framework, we integrated three taxon-based data sets including *Eleutherodactylus johnstonei* (this study, Leonhardt et al. 2019), *Eleutherodactylus portoricensis* (Barker et al. 2011) and *Eleutherodactylus antillensis* (Barker et al. 2012; Barker and Rodríguez-Robles 2017). In a first step, we compared molecular patterns (genetic diversity and differentiation, haplotype distribution) in the native and three introduced occurrence regions of the focus species *E. johnstonei* and reconstructed the invasion history based on mitochondrial D-loop sequences. For the two sister taxa we analysed genetic diversity, differentiation and haplotype distribution of the same mitochondrial D-loop fragment and compared them to the patterns uncovered in *E. johnstonei*.

Field sampling

Field sampling was carried out in the assumed native range of St Lucia (LCA, Feb – Mar 2020) and exotic ranges in Guadeloupe (GLP, Feb – Mar 2020) and in greenhouses

of European botanical gardens in Germany, Switzerland and the Netherlands (EUR, May – Aug 2018). Data sets for Colombia (COL) were established in a previous study (Leonhardt et al. 2019; field sampling between 2016 and 2018). We aimed at sampling a minimum of five individuals per sampling site in each of the four regions, covering a wide range of habitats (see Fig. 1 and Suppl. material 1 for details on sampling sites). Tissue samples for genetic analyses were acquired using minimally invasive toe clipping (Vences et al. 2012). After clipping the external phalanx, toes were disinfected with cotton pads soaked in 70% ethanol to prevent subsequent infections and individuals were immediately released afterwards. Samples were stored in 95% Ethanol and deposited in the tissue collection of the Museum of Zoology, Senckenberg Natural History Collections Dresden (MTD). As part of the respective national biocontrol procedures, individuals from Guadeloupe were not released but collected as scientific vouchers.

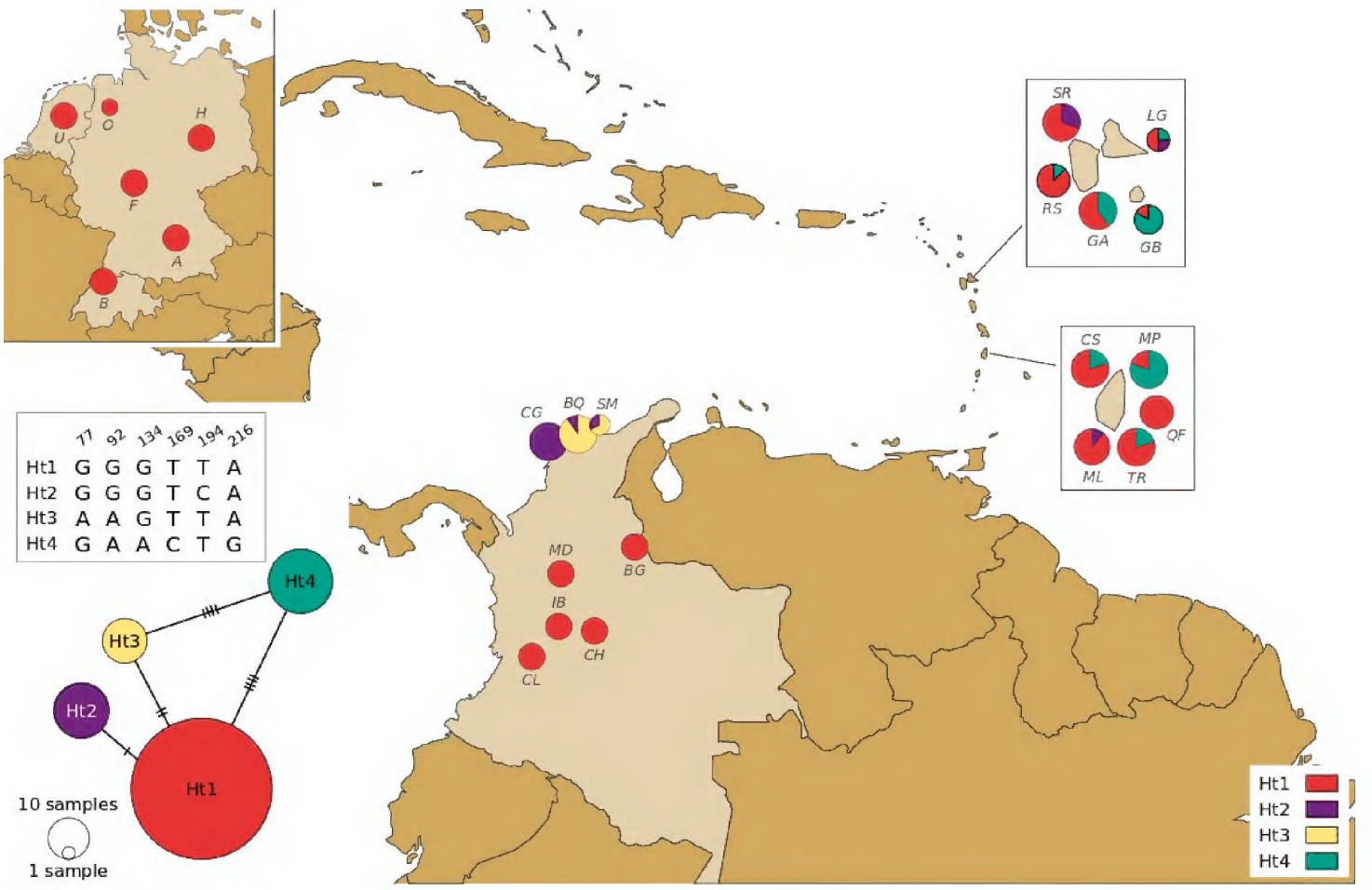

Figure 1. Haplotype distribution and network for *E. johnstonei* across native and exotic ranges. Bubble diagram of minimum spanning tree in the lower left shows interrelation between the four recovered haplotypes (Ht1, Ht2, Ht3, Ht4), circle size corresponds to sample size for respective Hts across the four regions, number of crossbars on connecting lines denote the number of polymorphic sites separating these haplotypes. Polymorphic sites are illustrated in the box above the haplotype network, numbers refer to positions in the alignment of the 235 bp D-loop fragment. The maps show the proportions of detected haplotypes at each population site, colours represent the haplotypes, circle size represents no. of samples; Europe: U – Utrecht, O – Osnabrück, H – Halle, F – Frankfurt, A – Augsburg, B – Basel, Colombia: CG – Cartagena, BQ – Barranquilla, SM – Santa Marta, MD – Medellin, BG – Bucaramanga, IB – Ibagué, CH – Chinauta, CA – Cali; Guadeloupe: SR – Saint Rose, RS – Rivière-Sens, LG – Le Gosier, GA – Grande Anse, GB – Grande Bourg; Saint Lucia: CS – Castries, MP – Morne Panache, QF - Quilesse Forest, ML – Morne Le Blanc, TR – Forest Ti Rocher.

These individuals were euthanized using commercially available toothache pain relief gel containing 20% Benzocaine and subsequently preserved in 70% Ethanol. Specimens are deposited in the collection of the Muséum national d'Histoire naturelle, Paris (MNHN) under collection numbers MNHN-RA-2021.0013 to MNHN-RA-2021.0062. For Saint Lucia, two specimens of each population were collected as reference vouchers and deposited at the Forestry Department of Saint Lucia.

Molecular data sets

The D-loop of the mitochondrial control region was chosen as a marker because it is the most polymorphic mitochondrial region (Stoneking et al. 1991; McMillan and Palumbi 1997; Bronstein et al. 2018) and mtDNA is more sensitive for the detection of population structure and history than nuDNA due to its higher mutation rate (Allio et al. 2017). Moreover, this marker has proven to yield robust patterns in previous studies on genetic structure in our target taxa (Leonhardt et al. 2019; Barker et al. 2011, 2012; Barker and Rodríguez-Robles 2017). For *E. johnstonei*, a total of 113 independent tissue samples from Saint Lucia (N = 48), Guadeloupe (N = 38) and Europe (N = 27) were used to generate mitochondrial (mt) haplotypes from partial sequences of the D-loop region (235 bp). These were complemented with 48 previously established sequences from Colombia using the same marker (Leonhardt et al. 2019). DNA isolation, PCR amplification of the D-loop fragment and sequencing were performed as described in Leonhardt et al. (2019). All sequences are deposited in NCBI GenBank under accession numbers OW993929–OW994041.

We performed a systematic NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) search for D-loop sequences of taxa that are congeneric with *E. johnstonei* and fulfil the following criteria: a) sufficient sample size (minimum N = 48, matching sample size for native range samples of *E. johnstonei*) and covering both native and exotic range in case of invasive taxa, b) available meta data (localities, etc.) provided in associated publications. Two datasets of *Eleutherodactylus portoricensis* (Barker et al. 2011) and *Eleutherodactylus antillensis* (Barker et al. 2012; Barker and Rodríguez-Robles 2017) met these criteria. Associated data are summarized in Table 1 (see Suppl. material 1 for more detailed information). For each species one sequence was used for a pairwise alignment with one *E. johnstonei* sequence, using BioEdit (Hall 1999), in order to define the respective partial sequence. Thus, the same partial D-loop sequence was used for all four species and in all subsequent analyses.

Molecular diversity and population genetic analyses

Sequence sets of each species (*E. johnstonei*, *E. antillensis*, *E. portoricensis*) were aligned using ClustalW multiple alignment within BIOEDIT Sequence Alignment Editor 7.2.5 (Hall 1999). Sites containing gaps were not considered for all subsequent analyses (assignment of haplotypes, parameters of molecular diversity and differentiation). All sequences were grouped by their respective sampling sites and regions as specified

Table 1. Molecular data sets of the three congeneric taxa.

Species	NCBI Genbank Accession no. & date	Distribution	Region	$N_{samples}$ / N_{sites}	Source
E. johnstonei	OW993929–OW994041	native	Saint Lucia (LCA)	48 / 5	this study, Leonhardt et al. 2019
		exotic	Guadeloupe (GLP)	38 / 5	
		exotic	Colombia (COL)	48 / 8	
		exotic	Europe (EUR)	27 / 6	
E. antillensis	JN385299–JN385583, KY636451–KY636487 (03/12/2020)	native	Western Puerto Rico (WPR)	139 / 28	Barker et al. 2012; Barker and Rodríguez-Robles 2017
		native	Eastern Puerto Rico (EPR)	64 / 13	
		native	Eastern Islands (EI)	67 / 14	
		exotic	Saint Croix (SCX)	37 / 5	
		exotic	Panama (PAN)	15 / 3	
E. portoricensis	HM229815–HM229958 (03/12/2020)	endemic	Puerto Rico – Cayey Mountains:	32 / 3	Barker et al. 2011
			Cerro de la Tabla (CAY-CT)	39 / 3	
			Carite State Forest (CAY-CS)	32 / 4	
			Puerto Rico – Luquillo Mountains:	15 / 1	
			El Yunque (LUQ-EY)	26 / 5	
			Pico del Este (LUQ-PE)		
			El Torro (LUQ-ET)		

in the source publications (see Table 1 and Suppl. material 1). To compare the genetic setup in the native vs. the exotic range of *E. johnstonei* and between *E. johnstonei* and congeneric sister taxa, the distribution and relatedness of haplotypes, as well as parameters of genetic diversity and population differentiation were analysed. For each of the three species, data on haplotype distribution within sites and regions was exported from DnaSP v6 (Rozas et al. 2017). Haplotype networks were generated with POPART (Leigh and Bryant 2015), using the Minimum Spanning network inference method. Haplotype networks were colour-coded by region to visualise the geographic distribution of haplotypes. Additionally, the distribution of haplotypes within native and exotic regions for the focal species *E. johnstonei* was mapped using QGIS 3.16.11 (QGIS Development Team 2021).

To address our hypothesis 1 (genetic bottlenecks) we estimated levels of molecular diversity within *E. johnstonei* across the four study regions (LCA, GLP, COL, EUR) and to address hypothesis 2 (genetic diversity differences between invasive and non-invasive species) additionally within native localities of all three sister taxa (*E. johnstonei*, *E. antillensis* and *E. portoricensis*). The following molecular diversity parameters were estimated based on pooled samples for an entire region, as well as for each locality within a region. The number of variable sites (s), the number of haplotypes (nHap) and how equally they are distributed (haplotype diversity, H_D), the average number of nucleotide differences between two sequences per site (nucleotide diversity, π) and the mean number of alleles per site (A) were analysed. All parameters were calculated with DnaSP v6 (Rozas et al. 2017), except for A, which was calculated with Arlequin v3.5.2.2 (Excoffier and Lischer 2010). Genetic differentiation of populations (i.e. localities) was assessed by pairwise F_{ST} values (fixation indices), calculated in DnaSP. Pairwise F_{ST} values estimate

the proportion of total genetic variation of two populations (γ diversity) between the two populations (β diversity) as opposed to the variation within the two populations (α diversity). Mann-Whitney-Wilcoxon tests were performed in R 4.0.2 (R Core Team 2021) to compare genetic diversity and differentiation of (1) populations in native regions vs. exotic regions of *E. johnstonei* and in (2) native populations of *E. johnstonei* vs. the two congeners. For Mann-Whitney-Wilcoxon tests we assumed that each locality represents one population, which means regions are compared by their average population-wide molecular diversity and pairwise population differentiation, respectively.

Results

Genetic makeup of *Eleutherodactylus johnstonei* in the native and introduced range

Populations of *E. johnstonei* show low molecular diversity and population differentiation across both native and exotic regions. Partial D-loop sequences (235 bp, 161 samples) across the whole sampled range feature only four haplotypes and six variable sites. Moreover, overall nucleotide diversity (0.0059) and haplotype diversity (0.5) are very low. A comparison of the three exotic (GLP: N = 38, COL: N = 48, EUR: N = 27) regions with the assumed native origin (LCA: N = 48) revealed that the latter did not exhibit the highest genetic diversity as originally hypothesised. In fact, molecular diversity within Guadeloupean populations was similar and even higher than in populations from Saint Lucia for all analysed parameters (see Table 2). European greenhouse populations, on the other hand, show clear signs of reduced molecular diversity as all analysed individuals show identical D-loop sequences corresponding to the haplotype Ht1. Considering the number of haplotypes (nHap), the number of variable sites (s), haplotype richness (H_R), haplotype diversity (H_D) and the mean number of alleles per locus (A), Saint Lucian populations are significantly more diverse than those from Colombia, while nucleotide diversity (π) is not significantly reduced in Colombia. Colombian populations are also more differentiated (F_{ST} = 0.443) than those from Saint Lucia (F_{ST} = 0.279) and Guadeloupe (F_{ST} = 0.206).

Geographic distribution of the four detected haplotypes across native and exotic ranges of *E. johnstonei*, as well as the haplotype network and variable sites defining the haplotypes, are illustrated in Fig. 1. The dominant haplotype Ht1 is present in 110 out of 161 samples (68%) and in all four regions. In all European localities and inland localities in Colombia, Ht1 is the only haplotype that was detected. Three additional haplotypes (Ht2, 17%; Ht3, 11%; Ht4, 23%) were detected with lower abundance. Ht2 is present both at the Colombian coast and on the two Caribbean islands, while it is much more common in the former. Ht3 is the only geographically unique haplotype, which was exclusively detected along the coast of Colombia. It is also the least abundant of all four haplotypes recorded. Ht4 is widespread across St. Lucia and Guadeloupe. Both islands share the same three haplotypes, while there is a clearer differentiation between haplotypes in exotic ranges outside the Caribbean.

Table 2. Parameters of molecular diversity and population differentiation for *E. johnstonei* in native and exotic regions. For each region no. of samples (N_{sam}) and no. of populations (N_{pop}) are given in brackets. nHap: no. of haplotypes (DNAsp), s: no. of variable sites (DNAsp), H_D: haplotype diversity (DNAsp), A: mean number of alleles per locus (Arlequin), π: nucleotide diversity (DNAsp), F_{ST}: average pairwise F_{ST} (DNAsp); for each region average values per population and total values for all samples (in brackets) are given; p-values of Mann-Withney-Wilcoxon tests testing for greater diversity and differentiation in St Lucia against the other regions are illustrated ($p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$), Mann-Withney-Wilcoxon tests were based on population averages.

Range (N_{sam} / N_{pop})	nHap	S	H_D	A	π	F_{ST}
St Lucia (48 / 5) native	1.8 (3)	2.6 (5)	0.26 (0.414)	1.011 (1.021)	0.004 (0.0067)	0.279
Guadeloupe (38 / 5) exotic	2.2 (3)	3.6 (5)	0.48 (0.553)	1.015 (1.002)	0.006 (0.008)	0.206
Colombia (48 / 8) exotic	1.25* (3)	0.75* (3)	0.11* (0.627)	1.003* (1.023)	0.001 (0.0047)	0.443
Europe (27 / 6) exotic	1** (1)	0** (0)	0** (0)	1** (1)	0** (0)	0

Table 3. Parameters of molecular diversity and population differentiation for native populations of *E. johnstonei* and sister taxa. For each species no. of samples (N_{sam}) and no. of populations (N_{pop}) are given in brackets. nHap: no. of haplotypes (DNAsp), s: no. of variable sites (DNAsp), H_D: haplotype diversity (DNAsp), A: mean number of alleles per locus (Arlequin), π: nucleotide diversity (DNAsp), F_{ST}: average pairwise F_{ST} (DNAsp); for each taxa average values per population and total values for all samples (in brackets) are given; Mann-Withney-Wilcoxon tests were based on population averages.

Species (N_{sam}/N_{pop})	nHap	S	H_D	A	Π	F_{ST}
E. johnstonei (48/5) successful alien	1.8 (3)	2.6 (5)	0.258 (0.414)	1.011 (1.021)	0.0038 (0.0067)	0.279
E. antillensis (270/55) successful alien	1.3 (15)	0.33 (12)	0.149 (0.546)	1.002 (1.055)	0.0007 (0.0027)	0.438
E. portoricensis (144/16) non-invasive, single-island endemic	5 (54)	6.1 (33)	0.806 (0.964)	1.028 (1.176)	0.0102 (0.0277)	0.457
	p(Ej < Ep) = 0.002**	p(Ej < Ep) = 0.015*	p(Ej < Ep) = 0.002**	p(Ej < Ep) = 0.008**	p(Ej < Ep) = 0.008**	p(Ej < Ep) = 0.04*
	p(Ea < Ep) = 5.58e-10***	p(Ea < Ep) = 3.88e-10***	p(Ea < Ep) = 1.05e-9***	p(Ea < Ep) = 7.34e-10***	p(Ea < Ep) = 4.09e-10***	p(Ea < Ep) = 0.18

Genetic diversity and population differentiation within and among species of the *Eleutherodactylus* model system

We found molecular diversity and population differentiation to be lowest in successfully colonising alien species. On average, all parameters estimated per native population (nHap, s, H_D, A, π, F_{ST}) are higher in *E. portoricensis* as compared to *E. johnstonei* and *E. antillensis*. This was also confirmed by Mann-Withney-Wilcoxon tests for all parameters except of F_{ST}, which indicate significantly lower population differentiation of *E. johnstonei*, but not of *E. antillensis*, as compared to *E. portoricensis* (see Table 3). Native populations of *E. johnstonei* and *E. antillensis* show similar diversity estimates, while populations of *E. antillensis* are slightly more differentiated (F_{ST}(Ej) = 0.279, F_{ST}(Ea) = 0.438).

Haplotype distribution and networks for all model taxa are visualised in Fig. 2. We found no significant spatial clustering of haplotypes in *E. johnstonei*. While Ht2 is the only haplotype that exclusively occurs in one region (COL), the most dominant haplotype Ht1 occurs across the entire range of investigation. A similar pattern emerged in the second successful alien, *E. antillensis*. Here, two dominant haplotypes comprise 78% of all samples. Geographic clusters are largely missing. However, a few less abundant haplotypes exclusively occur in a single region and the second most abundant haplotype clearly dominates western Puerto Rico. The single-island endemic *E. portoricensis* shows a markedly different pattern with clear geographic clustering. Populations of *E. portoricensis* are clearly divided into the two subregions, Luquillo and Cayey, which do not share any haplotype. Within those two subregions there are several private haplotypes (Hts exclusively occurring in a single population) and fewer haplotypes that are shared between populations (reflected by the lower F_{ST} value 0.478, see Table 3).

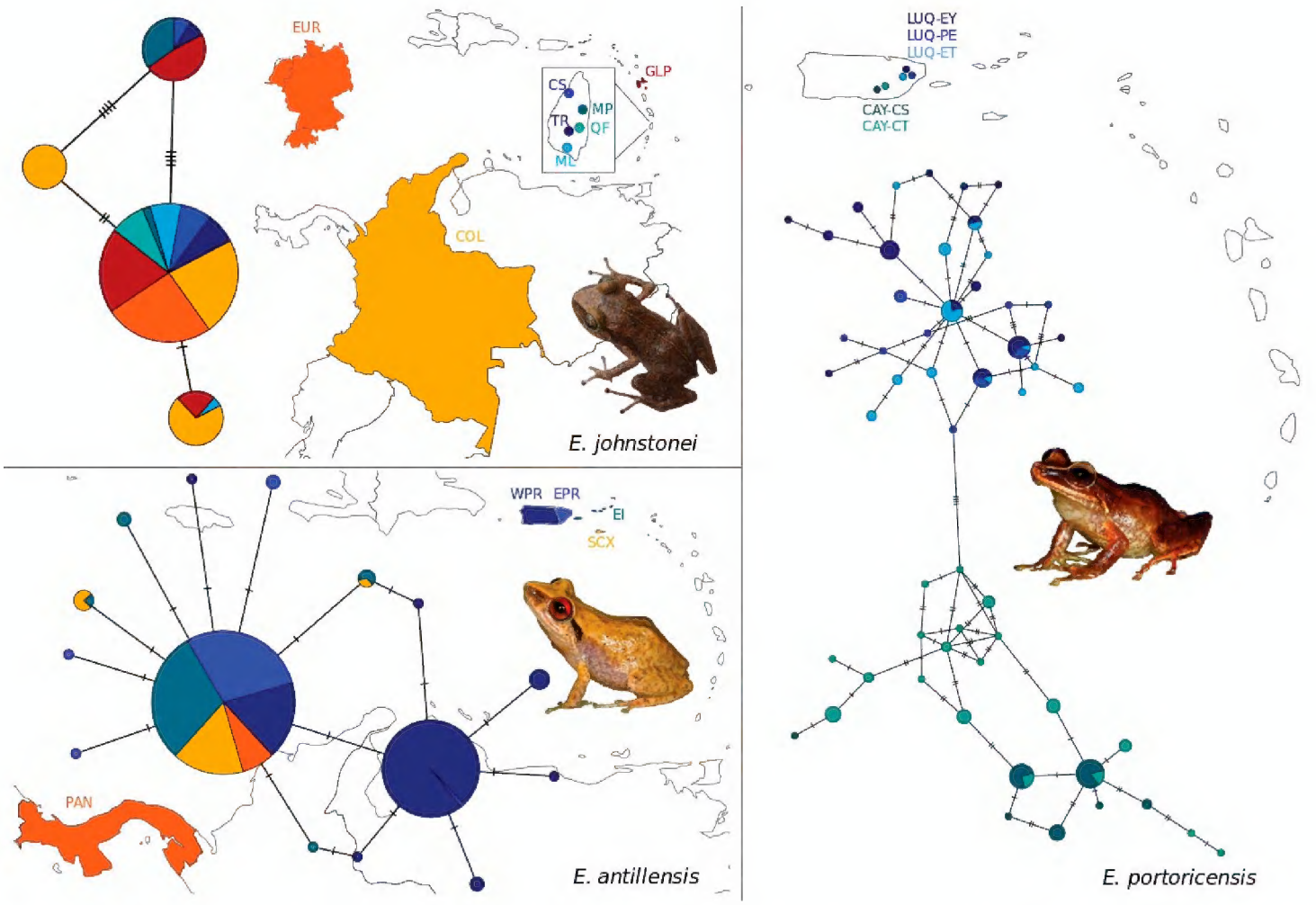

Figure 2. Comparative haplotype distribution and networks for all species of the *Eleutherodactylus* model system. Circle sizes correspond to respective sample sizes; pie chart colours correspond to respective populations. Yellow, orange and red correspond to exotic range populations in: EUR – Europe, COL – Colombia, GLP – Guadeloupe for *E. johnstonei*; PAN – Panama, SCX – Saint Croix for E. antillensis, blue and greenish colours represent native range populations (CS, QF, TR, MP, ML on Saint Lucia for *E. johnstonei*; WPR – Western Puerto Rico, EPR – Eastern Puerto Rico, EI – Eastern Islands for *E. antillensis*; LUQ-EY, LUQ-PE, LUQ-ET in the Luquillo Mountains and CAY-CS, CAY-CT in the Cayey Mountains on Puerto Rico for *E. portoricensis*). Photo sources: *E. johnstonei* - F. Leonhardt, *E. antillensis* - A. Lopéz, https://mir-s3-cdncf.behance.net/project_modules/max_1200/c1290514066123.5627cd91e1baf.jpg, *E. portoricensis* - A.D. Colón Archilla, https://alfredocolon.zenfolio.com/p973584972/h21cf17fc#h21cf17fc.

Discussion

The Caribbean features America's most extensive Cretaceous and Cenozoic oceanic-continental tectonic zone and it has the majority of the active volcanic centres of the New World (Donelly 1989). Therefore, the region represents an ideal model to test (island)biogeographic theories and their molecular basis (e.g. Hedges et al. 1992; Losos and Schluter 2000; Vellend 2003; Dugo-Cota et al. 2019). However, the role of alien taxa in shaping biogeographic patterns in this region has only recently been studied (Helmus et al. 2014). Here we established the first comprehensive molecular data set covering both the native and exotic range of the most widespread amphibian species with a Caribbean origin, *Eleutherodactylus johnstonei*. In contrast to what we expected, we detected comparatively low levels of genetic diversity and population differentiation in the species' assumed native range, St. Lucia. We observed similar genetic patterns in introduced populations on the islands of Guadeloupe. Exotic populations outside the Caribbean, however, were genetically impoverished, indicating marked founder effects. As in *E. johnstonei*, the invasive congeneric *E. antillensis* showed comparably low genetic diversity in its native range. In stark contrast to this pattern, we found marked inter-population differentiation and higher overall molecular diversity in the non-invasive congener *E. portoricensis*.

The genetic patterns observed in exotic populations of *E. johnstonei* (see Fig. 1) mirror respective introduction histories in the three regions. Only a single haplotype (Ht1 *sensu* Leonhardt et al. 2019) is present in European greenhouse populations. Since Ht1 is also the dominant haplotype in populations from Guadeloupe, our results support a single introduction event in 1993, when the Botanical Garden of Basel received a plant shipment from Guadeloupe (H. Schneider pers. comm.) that likely contained the founder individuals. Additional populations were subsequently established through deliberate exchange between the European botanical gardens. Colombian populations of *E. johnstonei* show higher levels of both genetic diversity and inter-population differentiation, which supports the previously proposed two to three independent introduction events (Leonhardt et al. 2019). All Colombian inland populations exhibit the dominant haplotype Ht1 and were likely derived from a single introduction to Bucaramanga (Ortega et al. 2001; Leonhardt et al. 2019). The native range populations from St. Lucia, as well as populations from Guadeloupe, are possible sources of this introduction. For coastal populations in Colombia, two scenarios are possible: (1) In two independent introductions, as previously hypothesised in Leonhardt et al. (2019), Ht3 was introduced to Barranquilla and Ht2 to Cartagena. Individuals subsequently spread along the coast via jump dispersal, as described in Ernst et al. (2011), thereby establishing the Santa Marta population and introducing Ht2 into the Barranquilla populations. In this scenario, St Lucia or Guadeloupe are possible sources of the introduction to Cartagena (Ht2) and Ht3, introduced to Barranquilla, either originates from an un-sampled Caribbean island or was missed in our Caribbean samples due to its rarity. (2) A single introduction to Barranquilla from a Lesser Antillean source population containing both Ht2 and Ht3, and subsequent distribution

to Santa Marta and Cartagena. Disentangling these competing scenarios would require additional sampling in yet un-sampled Caribbean localities, as well as the use of higher resolution molecular markers. Guadeloupean populations of *E. johnstonei* do not only show higher genetic diversity, but also higher connectivity between populations revealed by spatial haplotype distributions. These differences in genetic patterns between Caribbean (GLP) and non-Caribbean (COL, EUR) introduced populations are mirrored in distribution patterns. While Colombian and European populations are spatially confined to urban and peri-urban habitats and greenhouses, Guadeloupean populations occupy a wider range of (mainly disturbed) habitats, resulting in a less patchy distribution (pers. obs., Kaiser 1997; Breuil 2002). These differences are likely caused by two main factors: (1) Guadeloupe's proximity to native range populations that allowed for several, possibly still ongoing, independent introductions and (2) the general ecosystem resemblance among the Caribbean islands as compared to introduction localities in non-Caribbean regions. Although observed genetic patterns revealed by the analyses of the mitochondrial D-loop fragment corroborate previously assumed invasion histories in the three exotic regions, additional marker systems, such as SNPs or microsatellites are desirable to add more power to the analytical framework (e.g. Guillemaud et al. 2010).

Genetic diversity and inter-population differentiation in *E. johnstonei*'s assumed origin St. Lucia (Censky and Kaiser 1999) was not higher than in Guadeloupe. Although novel molecular evidence was recently provided (Yuan et al. 2022), the actual origin has remained speculative to date (Kaiser 1997; Lescure 2000; Yuan et al. 2022). Our data allow two possible scenarios: (1) The native range of *E. johnstonei* is larger than previously assumed and includes several Caribbean islands. This would be in line with Yuan et al. (2022) who identified two mitochondrial lineages that are restricted to the eastern and western Lesser Antillean islands, respectively. The authors identified Montserrat as the most likely origin of the western clade and this may also be the source of our unique Colombian coastal haplotype (Ht3). The eastern clade, including the islands of St Lucia and Guadeloupe, was proposed to be the source of introduced populations on Jamaica, Curaçao, Trinidad and the Venezuelan mainland. While Yuan et al. (2022) consider *E. johnstonei* to be introduced to St Lucia, their sampling in this locality was limited and persuasive alternative origins of the eastern clade, are missing. Therefore, we propose an alternative scenario to be tested: (2) Rapid human-induced environmental change on St. Lucia resulted in habitat loss (Mycoo et al. 2017) and led to the extinction of local populations and therefore the loss of unique haplotypes that still persist in the introduced range (e.g. Ht3 in the coastal Colombian populations). If this scenario is true, exotic range populations safeguard genetic diversity that was lost in the native range. This raises the question of the role of these non-native populations in diversity conservation (compare Jones 2003; Osborne et al. 2013). A combination of advanced molecular approaches (McCartney et al. 2019; North et al. 2021) and assembly of existing mitochondrial and nuclear markers (this study, Yuan et al. 2022) across the entire (native and exotic) range of the species, as well as comparisons of the historical and current distribution of *E. johnstonei* and detailed niche models (Leonhardt et

al. in prep.) would allow to further test this assumption. We can also not fully rule out the possibility that we missed unique and rare haplotypes in our sampling scheme and that intensified sampling would eventually yield these "missing" haplotypes.

Despite the differences between native and introduced populations, overall genetic diversity in *E. johnstonei* is comparatively low and matches that of the congeneric *E. antillensis* (Barker et al. 2012; Barker and Rodríguez-Robles 2017). Investigated populations of *E. antillensis* feature only two dominant haplotypes and their distribution suggests a high connectivity among native populations in Puerto Rico. The native ranges of both *E. johnstonei* and *E. antillensis* are comparatively small and this has previously been suggested to explain low levels of molecular diversity in the latter (Barker et al. 2012; Barker and Rodríguez-Robles 2017). Our analyses of the restricted (235 bp) D-loop fragment seem to corroborate this assumption at first sight. However, the recovered patterns in the range-restricted and endemic *E. portoricensis* are in stark contrast to this observation. We found significantly higher levels of genetic diversity and spatial differentiation (geographic clusters), despite its small native range (this study, Barker et al. 2011). This may partially be explained by the habitat preferences and the spatial configuration of the habitat template occupied by *E. portoricensis*. The single island endemic is restricted to two mountain ranges (Luquillo and Cayey) that are separated by the Caguas river basin. The basin represents a barrier for the montane rainforest specialist and likely promoted the differentiation of two mitochondrial lineages (Velo-Antón et al. 2007; Barker et al. 2011). *E. antillensis*, on the other hand, is broadly distributed throughout the lowland, up to middle elevation habitats on Puerto Rico. Accordingly, the genetic structure is far less fine-scaled and mainly marks an east-west clade (Barker et al. 2012). The small (616 km^2) island of St. Lucia features only one central volcanic ridge (Mount Gimie, 958 m a.s.l.) and is thus geographically far less structured than Puerto Rico. This likely promotes gene flow that explains the observed genetic patterns in the generalist *E. johnstonei*, one of the most ubiquitous taxa in the herpetofauna of the island (Daltry 2009). Geological processes in the native ranges and the mode and timing of introductions in non-native localities are likely the main drivers shaping the genetic patterns detected in our *Eleutherodactylus* model system. While this is not unexpected, it cannot explain the invasion success of our alien amphibian model taxa.

Although it is commonly assumed that high intra-population genetic diversity promotes the adaptive capacity of a species and therefore correlates with invasion success, empirical data does not seem to support this notion (Harrison and Mondor 2011; Rollins et al. 2013; Trucchi et al. 2016). Successful alien amphibians investigated in our study (*E. johnstonei* and *E. antillensis*) show low genetic diversity in their native ranges as compared to an endemic congener. Although the single mitochondrial marker used here unarguably yields robust and ecologically interpretable results, we acknowledge the fact that employing molecular approaches with a higher resolution and coverage of several genomic regions, e.g. genotyping-by-sequencing approaches (Forsström et al. 2017; McCartney et al. 2019) or whole-genome re-sequencing (North et al. 2021) may provide slightly deviating results. However, this requires systematic testing, ideally within an identical framework.

The existing data strongly support the relevance of ecological and anthropogenic factors that drive the invasion process in our target taxa and explain the establishment success of our focus taxon *E. johnstonei*. These include: (1) Increased continuous propagule pressure (Simberloff 2009), i.e. reoccurring introduction events increase the statistical probability of a successful establishment (Leonhardt et al. 2019). (2) Exotic populations establish in specific microhabitats that resemble conditions in the native range habitats (greenhouses, urban and peri-urban gardens and tree nurseries) and therefore restrict the expansion potential (Ernst et al. 2011; Leonhardt et al. 2019). (3) Pre-adaptations that have been shown to favour successful invasions, such as direct development and therefore independence of aquatic reproduction habitats (van Wilgen and Richardson 2012; Allen et al. 2017) and the occurrence in human-altered habitats in the native range (Hufbauer et al. 2012), which further facilitates trans-location. Together, these factors may override potential impacts of genetic diversity and explain why genetic diversity *per se* does not translate into higher invasion success (Harrison and Mondor 2011; Rollins et al. 2013; Trucchi et al. 2016).

Frequent environmental disturbance causes a decrease of genetic diversity in various taxa (Banks et al. 2013), but selects for increased environmental tolerance (Leidinger et al. 2021) and phenotypic plasticity (Meyers et al. 2005), thereby hampering local adaptation (Kawecki and Ebert 2004). These conditions are met on many of the Caribbean islands, including St. Lucia that has been exposed to volcanic activity, frequent and reoccurring hurricane events and sea level changes (Government of Saint Lucia 2002; Mycoo et al. 2017). This likely contributed to the observed genetic patterns in the native populations and resulted in phenotypic plasticity, which is reportedly high in the entire genus *Eleutherodactylus* (Hoffman and Blouin 2000; Woolbright and Stewart 2008) including *E. johnstonei* (Ovaska 1991; Kaiser 2002). At the same time, populations on small islands, such as those of *E. johnstonei* on St Lucia, may have contributed to persistent inbreeding spanning generations. Thus deleterious alleles can be excluded from the gene pool, resulting in reduced genetic diversity and increased resistance to continuous inbreeding (Crnokrak and Barrett 2002). The detected genetic patterns reflect these assumptions and provide support for the pre-adaptation hypothesis explaining the establishment success of *E. johnstonei* despite low genetic diversity.

Conclusion

Our empirical results add to an increasing body of evidence showing that successfully invasive species are not genetically more diverse or structured than their non-invasive congeners (Gaither et al. 2013; Rollins et al. 2013; Trucchi et al. 2016; Baltazar-Soares et al. 2017; Wellband et al. 2017). Genetic variation, assessed by standard molecular markers, rarely affects invasion success (reviewed in Dlugosch et al. 2015) and rapid adaptation is not limited by low genetic variation (Bock et al. 2015). If molecular processes alter the invasion process, it is likely to be through mediating response plasticity under epigenetic control (DNA methylation, Hawes et al. 2018) or through functional pre-adaptations detectable only through functional genomics (McCartney et al. 2019).

Focusing on anthropogenic drivers and ecological factors that provide simpler explanations is likely more relevant from a practitioner's point of view and will be more effective in guiding control and management decisions.

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Supplementary material 1

Detailed information on all populations of the three congeneric taxa used in the molecular data sets of this study

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Data type: Occurences.

Explanation note: For each population of the three taxa, the following details are given: region, site, coordinates (if available), NCBI GenBank Accession numbers, source publication.

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